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Hetaerina giselae sp. n. (Odonata: Calopterygidae) from Southeastern Brazil, with larval and adult diagnosis

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Abstract. *Hetaerina giselae* sp. n. is described and illustrated based on specimens collected at Pico do Itambé State Park, Minas Gerais, Brazil. Integration of morphological and molecular data (COI) supports the recognition of the new species. The new species can be separated from its morphologically closest congeners, such as *Hetaerina longipes* Hagen in Selys, 1853, and *Hetaerina westfalli* Rácenis, 1968, by a combination of characters of the male anal appendages, female intersternite, and general anatomical features. In addition, we present the description of the larva, highlighting the main morphological differences that distinguish it from adults and other known larvae of the genus.

Keywords. Zygoptera, damselfly, description, dragonfly, new species, South America

Resumo. *Hetaerina giselae* sp. n. é descrita e ilustrada com base em espécimes coletados no Parque Estadual do Pico do Itambé, Minas Gerais, Brasil. A integração de dados morfológicos e moleculares (COI) apoia o reconhecimento da nova espécie. A nova espécie pode ser separada das espécies mais morfologicamente próximas, como a *Hetaerina longipes* Hagen in Selys, 1853 e *Hetaerina westfalli* Rácenis, 1968, por uma combinação de caracteres dos apêndices anais dos machos, intersternito das fêmeas e características corporais gerais. Além disso, apresentamos a descrição da larva, destacando as principais diferenças morfológicas que a distinguem dos adultos e de outras larvas conhecidas do gênero.

Palavras-chave. Zygoptera, donzelinha, descrição, libélula, nova espécie, América do Sul

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Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Introduction

The genus *Hetaerina* Hagen in Selys, 1853, is distributed from southern Canada to northern Argentina, with the greatest diversity found in South America (Garrison, 1990). There are 37 species recognized so far (Paulson et al., 2025), with nearly half of them (18 spp.) occurring in Brazilian territory (ICMBio, 2025).

Before the review conducted by Garrison (1990), the genus was challenging due to their close morphological similarity. Although the shape of male cerci remains a primary diagnostic feature, recent studies have shown that these structures can exhibit significant interspecific convergence. This morphological overlap can hinder accurate identification and has led to the discovery of cryptic species within the genus, even when multiple lines of evidence are applied (Vega-Sánchez et al., 2019, 2020b). Consequently, a modern taxonomic approach integrating adult morphology, larval characteristics, and molecular data is often necessary to resolve complex species groups and describe new taxa. When dealing with females, the complication is even greater, as there are few characteristics to identify these individuals, such as body coloration patterns and intersternite morphology (Garrison, 1990; Garrison et al., 2010).

In addition to adults, approximately half of the larvae of the genus *Hetaerina* have been described (Garrison et al., 2010; Pessacq & Muzón, 2004). However, some larvae were identified based on their coexistence with adults in the same locality, a method that lacks the certainty provided by rearing larvae until emergence or through molecular association. Therefore, greater sampling efforts and more rigorous association methods are essential to accurately identify and describe the remaining juvenile stages of the group.

Therefore, the objective of this study was to describe the male, female, and larva of a new species based on morphological and genetic data.

Material and methods

Sampling site

The specimens were collected in Pico do Itambé State Park (PISP), located in the upper region of the Jequitinhonha Valley in the central part of the Espinhaço Range in Minas Gerais, Brazil (-18.387444° S, -43.303972° W, 1178 m a.s.l.), an area of rupestrian fields/rocky outcrop vegetation associated with Atlantic Forest fragments. Collections were performed between May 2024 and February 2025 under ICMBio/SISBIO and IEF-MG licenses (IEF-MG: #017/2024; SISBIO: #93039-1).

Specimen collection

Larvae were collected, transported, and reared following the methods recommended by Cezário et al. (2021). The larvae were collected using a 4 mm² mesh hand sieve with a diameter of 40 cm, tweezers, and trays. After collection, they were placed in separate plastic cups (300 mL) filled with approximately ¼ of the water and substrate (support) for transport. In addition to the larvae, an exuvia was collected, which was found next to the adult, as it had just emerged.

Adults were collected using an entomological net, and the ad libitum methodology was used to further explore the sampled areas, which were close to the areas where the larvae were found. Collections were performed at different times of the day, with at least one hour of collection per location, always between 8:00 a.m. and 2:00 p.m. (Vilela et al., 2020). The adults were placed in entomological envelopes with proper identification (collection site, date, and collector), where they remained for eight hours to empty their digestive tract, and were then submerged in 70% alcohol for sacrifice, where they remained for a minimum period of eight hours (Garrison et al., 2010).

Image processing

All illustrations were made using a Galaxy Tab S9 FE+ on the Infinite Painter app, and images were made using a Digilab DI-106T stereomicroscope and assembled using Inkscape software (https://inkscape.org/pt-br/).

Molecular analysis

To determine the phylogenetic position of the new species, we sequenced the COI gene, a commonly used marker for delimiting and positioning odonates (e.g., Koroiva & Kvist, 2018). Total genomic DNA was extracted from four specimens [two males (Z028, Z027), one female (Z028, in copulation with male Z028), and one larva (RK97)] using the DNA Blood & Tissue Kit (Qiagen, Germany). Polymerase Chain Reaction (PCR) amplification of the COI gene was performed using OdoF1_t1 and OdoR1_t1 primers under the conditions described by Vilela et al. (2019). The PCR products were bidirectionally sequenced using an ABI 3130 Genetic Analyzer (Applied Biosystems).

We used GENEIOUS PRIME v.2025.2.2 (Kearse et al., 2012) to assess sequence quality by comparing the strands with their respective chromatograms and to assemble and edit the sequences as needed. Ninety-six "COI" sequences from the "Folmer region" (see Koroiva et al., 2018) of *Hetaerina* species available in GenBank (accessed November 17, 2025) were included (Table S1). All COI sequences available in GenBank and all sequences used by Standring et al. (2022) were included, except those from Vega-Sanchez et al. (2020a). *Calopteryx* spp. sequences were used as outgroups (Table S1).

For phylogenetic analysis, sequences were aligned using Clustal Omega 1.2.2 (Sievers et al., 2011), a module implemented in GENEIOUS PRIME v.2025.2.2. Alignment was used to calculate pairwise genetic distances in GENEIOUS PRIME v.2025.2.2. For tree construction, we used the GTR+I+G nucleotide substitution model, as suggested by Abadi et al. (2019). A maximum likelihood (ML) tree was constructed using RAxML v.8.2.11 (Stamatakis, 2014) with 1000 bootstrap replicates. Our sequences were deposited in GenBank (NCBI) under accession numbers PX559981-PX559984.

Morphological assessment

Wing nomenclature follows Riek & Kukalová-Peck (1984) and abdominal appendage nomenclature follows Garrison (1990). Morphological terminology followed Novelo-Gutiérrez (2009). All measurements were recorded in millimeters (mm). Measurements were performed using ImageJ software (https://imagej.net/ij/).

Abbreviations for the larvae: AL, abdomen length (without caudal lamella); Pfl, prothoracic femur length; MsfL, mesothoracic femur length; MtfL, metathoracic femur length; TL, total length; S1–S10, abdominal segments.

All specimens (larvae and adults) were deposited at collection "Coleção Biológica de Vespas Sociais" (CBVS, https://vespas.ifs.ifsuldeminas.edu.br/), from the Sul de Minas Federal Institute, Inconfidentes, Minas Gerais, Brazil.

Results

Hetaerina giselae sp. n. Dias-Oliveira, Koroiva & Vilela

Figures 1–7.

Material examined

Holotype ♂ and allotype ♀ Brazil, Minas Gerais State, Pico do Itambé State Park, Fumaça Waterfall (-18.454639° S, -43.334500° W, 817 m a.s.l.), 25-v-2024, leg. Simões, M.L.S. Deposited at collection CBVS, from the Sul de Minas Federal Institute, Inconfidentes, Minas Gerais, Brazil.

Paratypes. 63♂♂ 2♀♀ (including the specimens used for DNA analysis): Brazil, Pico do Itambé State Park: Campina: 2♂♂ (-18.493194° S, -43.364222° W, 1170 m a.s.l.), 02-xii-2024, 24-ii-2025; Água Santa Waterfall: 3♂♂ (-18.439750° S, -43.300139° W, 841 m a.s.l.), 27-v-2025; Fumaça Waterfall: 19♂♂1♀ (-18.454639° S, -43.334500° W, 817 m a.s.l.), 25–26-v-2024, 22-ii-2025; Neném Waterfall: 13♂♂1♀ (-18.422111° S, -43.308056° W, 1075 m a.s.l.), 10-ix-2024, 01-xii-2024, 21-ii-2025; Rio Vermelho Waterfall: 20♂♂ (-18.387444° S, -43.303972° W, 1178 m a.s.l.), 26-v-2024, 09–12-ix-2024, 29-xi-2024, 03-xii-2024, 23-ii-2025; Tropeiro's trail: 6♂♂ (-18.444278° S, -43.336556° W, 947 m a.s.l.), 21-ii-2025. Deposited at collection CBVS, from the Sul de Minas Federal Institute, Inconfidentes, Minas Gerais, Brazil.

Etymology

Named *giselae* (noun in the genitive case) after Gisele Alves de Oliveira, the mother of TMDO.

Holotype

Head. Labrum mostly yellow. Postclypeus and anteclypeus black. Vertex, postocular lobe, rear of the head and antennae, black (Figure 1a).

Thorax. Pronotum black, propleuron brown. Pterothorax predominantly dark metallic reddish, especially on the mesepisternum and mesepimeron. Pale humeral suture in the form of a thin line, more pronounced in the proximal portion; second lateral stripe present, pale and narrow, following the posterior portion of the mesepimeron; metepimeron pale, except for a thick dark medial spot.

Wings. Fw hyaline with a small reddish-brown apical spot, without pterostigma; basal red spot extending two cells beyond the quadrangle and bounded anteriorly by the RA and anal margin; venation within the red spot, white pruina along the transverse veins, and remainder black. Hw hyaline with brown basal spot, reaching 16 antenodal veins extending basally to the quadrangle and extending anteriorly slightly beyond the subcosta, but not reaching the costa, becoming weaker in the final portion; venation black, except for the brown venation in the basal area; costa black; apical spot reddish-brown, darker than the spot on the hindwing, without pterostigma (Figure 1c).

Abdomen. Matte black, with reddish basal rings in S1–S2 and S2–S3.

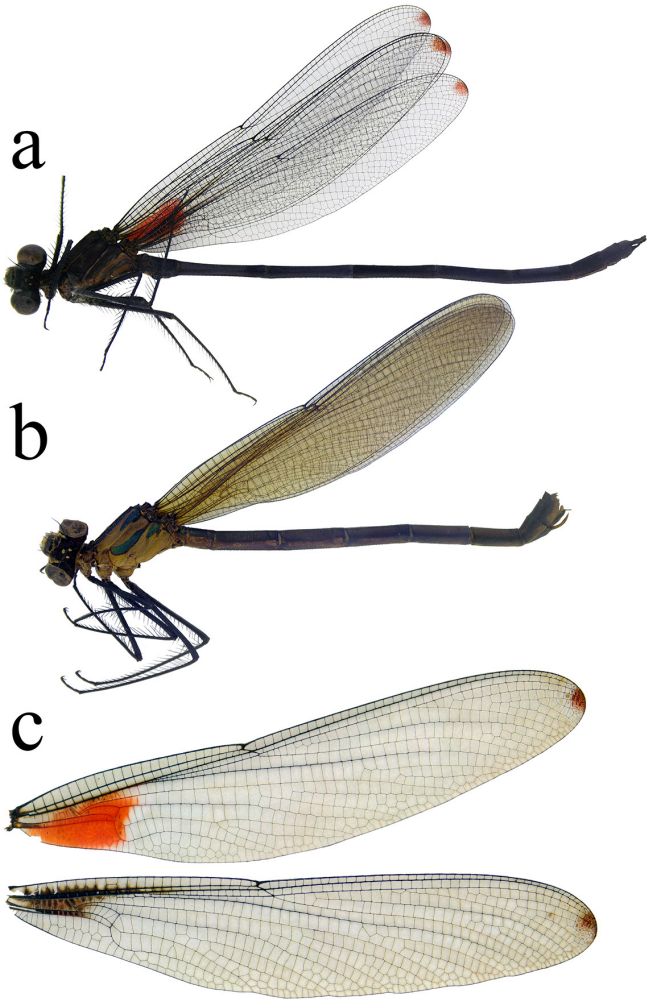

Figure 1. (a) Holotype male habitus; (b) paratype female habitus; (c) paratype male wings, Fw (above) and Hw (below).

Anal appendages. Cerci black; distal fossa well defined (Figure 2a,b,c); prominent transverse ridge connecting to the upper ridge at an almost right angle (Figure 2a,b,c,d); medial lobe with a "B-shaped" edge, almost connecting to the elevated transverse ridge (Figure 2c); basal portion lacking any tubercle-like structure; paraprocts with a small basomesal process, distal process approximately 0.49 of the length of the upper appendage, ending in a truncated, rounded apex (Figure 2b,c); in lateral view, with a tuft of hairs at the base and a slightly upward-curving apex (Figure 2e).

Measurements [mm]. Hw 28, abdomen 37.7.

Variation in paratypes

Color of mesepisternum metallic green (10%), reddish green (14%), non-metallic black (8.8%) when illumination is perpendicular to the axis of thorax; metepimeron dark brown, except for a thick dark medial spot (25%); both wings with a red basal spot (4.4%), very small Hw basal spot, almost non-existent (3%); medial lobe, with very subtle elevation, with an almost imperceptible B-shape (7%).

Female allotype

Head. Labrum yellow; mandible yellow; postclypeus dark brown and anteclypeus yellow; antefrons with a thin yellow line that is expanded in the center and extends laterally to the pedicel, forming a smooth M-shape; postfrons black, as is the rest of the upper part of the head; ocelli yellow (Figure 1b).

Thorax. Prothorax: iridescent green pronotum, light brown propleuron. Pterothorax predominantly brownish, with iridescent green stripes; thick green antehumeral stripes on both sides; mesepisternum with a small stripe, arising at the posterior region and tapering off at ½ of the mesepisternum; mesepimeron with a spot that arises narrowly at the interpleural suture and expands anteriorly, "comma-shaped", almost reaching the posterior edge of the mesinfraepisternum; metepimeron with a small ovaloid green spot at the posterior portion. Intersternite digitiform, linear, with a smooth anterior shoulder, almost non-existent, followed by a slightly rounded posterior branch (Figure 2f). Coxae pale yellow, legs black, spines larger than the space between them, and decreasing in size towards the apex.

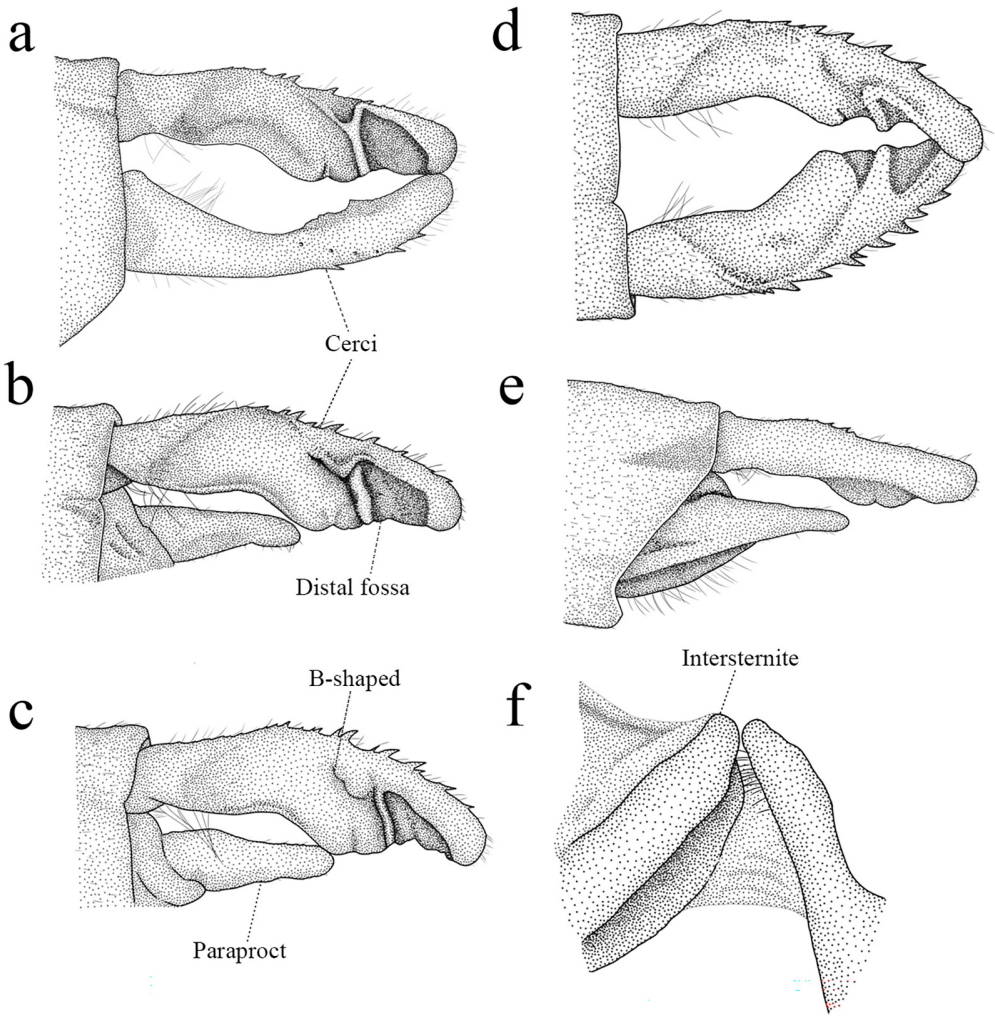

Figure 2. Holotype male cercus in a sequence of rotation (a–e): (a) latero-dorsal view; (b) mediodorsal view; (c) dorso-lateral view; (d) dorsal view; (e) lateral view; (f) female intersternite.

Wings. Fw and Hw hyaline, slightly tinged with pale yellow, with a darker yellowish from the base to the distal end of the quadrangle and in the costal and subcostal spaces up to the subnodus. Pterostigma absent.

Abdomen. S1–S2 dorsally metallic green, laterally yellow, ventrally dark brown; S3–S5 dorsally black, laterally and ventrally brown. S6–S10 dorsally and laterally black, ventrally brown. Cerci and ovipositor brown.

Variation in paratypes

Mesepisternum and mesepimeron predominantly metallic green (33,3%)

Adult diagnosis

The new species is morphologically close to *Hetaerina westfalli* Rácenis, 1968 (a predominantly northern Brazilian species) in terms of body size and appendage morphology, except for the lack of a pterostigma. However, our molecular analysis recovered *H. giselae* sp. n. as the sister taxon of *Hetaerina longipes* Hagen in Selys, 1853, with good phylogenetic support (83%, Supplementary Figure 1). The average distance between the COI sequences of *H. giselae* sp. n. and *H. longipes* was 6.92% (range 6.43–7.39%). The analysis also showed that *H. westfalli* was placed near other species, such as *Hetaerina indeprensa* Garrison, 1990, *Hetaerina curvicauda* Garrison, 1990, and *Mnesarete williamsoni* Garrison, 2006. Our new species can be separated from *H. westfalli* and *H. longipes* by the following character combination: a wider and deeper distal fossa, occupying at least ¾ of the apical portion of cercus, which is nearly the same width as medial lobe (narrower fossa, occupying less than ½ of the apical portion of cercus in *H. westfalli*, tapering after medial lobe not abrupt; distal fossa and apical portion of cercus narrow, with abrupt tapering after medial lobe); medial lobe with a "B-shaped" edge, in dorsal view almost like a cleft (Figure 2a) (medial lobe with rounded or nearly triangular edge in *H. westfalli*; medial lobe digit-shaped, pronounced laterally, not forming a "B-shaped" edge); lacking a tubercle-like structure on the basal portion of cercus (with a tubercle-like structure on the basal portion of cercus on *H. westfalli*; basal tubercle also absent in *H. longipes*); in lateral view, cercus nearly straight, with evident "B-shaped" medial lobe (in lateral view, cercus nearly straight, with evident medial lobe and basal tubercle in *H. westfalli*; in lateral view, cercus slightly curved downwards after medial lobe, which is also visible in *H. longipes*).

Description of the larva
(3♂♂, same data as the type material)

Dark brown larva, slender, with light brown legs with small dark spots along the femur and tibiae. Light brown caudal gills with a darker basal portion (Figure 3a).

Head. Dark brown, 0.42 times wider than long, wider than the thorax and abdomen, with a prominent protuberance and rounded posterolateral angles (Figure 3b,c). Antennae long and filiform, 3-segmented, light brown,

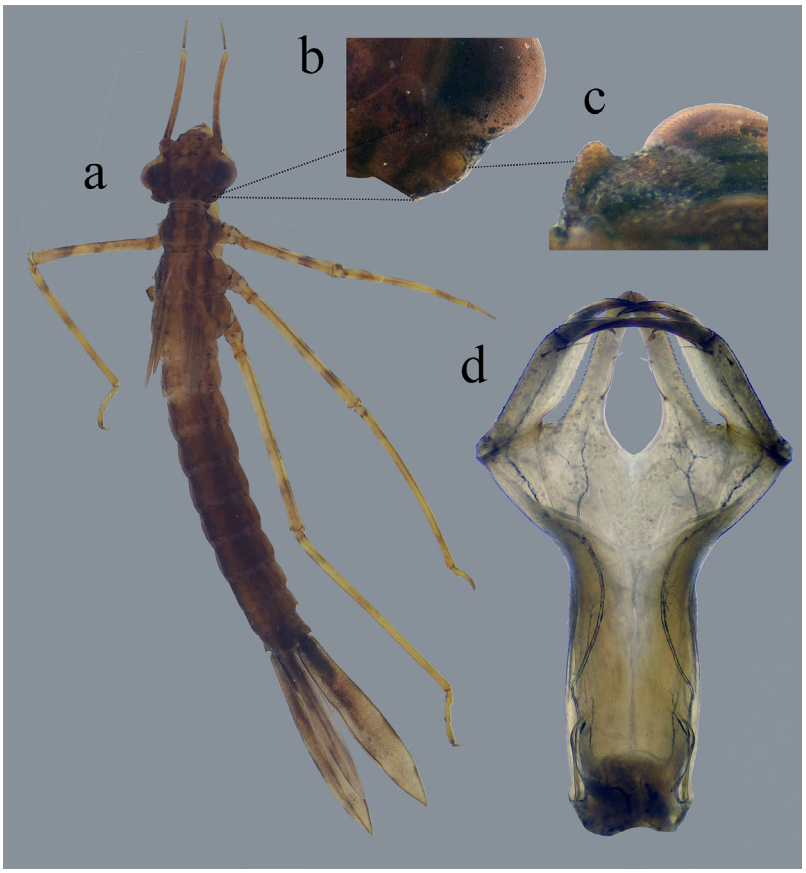

Figure 3. (a) Larval habitus. (b) detail of cephalic lobe; (c) posterolateral protuberance; (d) dorsal view of prementum.

darker at the basal portion of the segments; 1st antennomer the longest; proportional length of antennomeres: 1, 0.33, 0.19; compound eyes rounded. Mandible formula: L 12345 0 a($m^{1,2,3,4,5,6}$)b / R 12345 y a (Figure 4a). Prementum distinctly petiolated, proximal portion with straight lateral margins 1.7 longer than wide, shallow apical cleft, about 18% of the maximum width of the prementum and 26% of the total length (Figure 3d); two setae on each margin of the cleft; apex of the prementum with fine crenulation (Figure 5b) Labial palp with a basal setae and a palpal setae, three teeth, all smaller than the mobile hook, the inner one being the smallest and the middle one the largest, with lengths proportional to: 0.4, 1, 0.84; movable hook as long as the outer margin of the palp, with acute apex (Figure 5a).

Thorax. Dark brown, with darker spots on the sutures; legs slightly lighter than the body, with a few scattered dark spots; the tip of the metathoracic tarsi almost reached the apex of the lateral gills when extended.

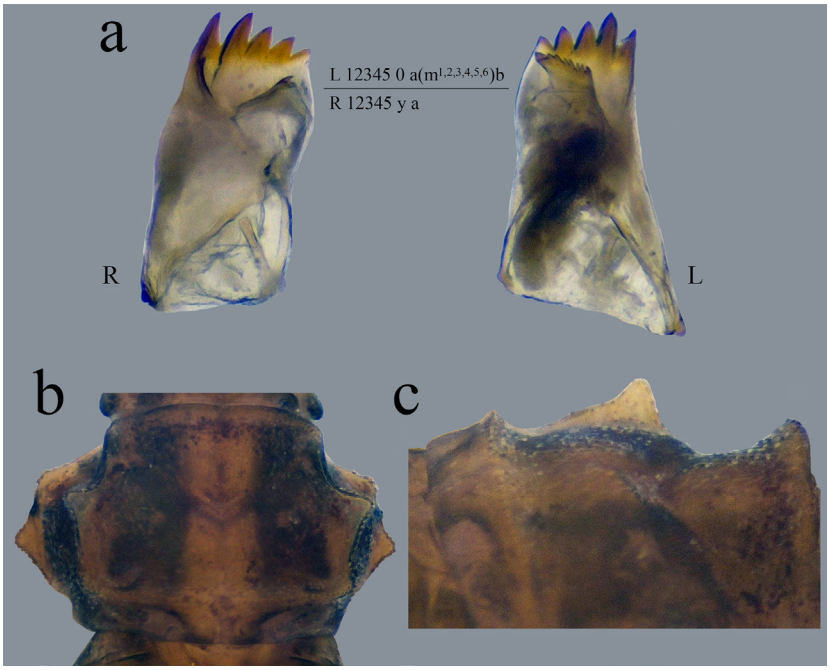

Figure 4. (a) Mandibles; (b) dorsal view of pronotum; (c) dorso–lateral view of pronotum, showing the pronotal protuberances.

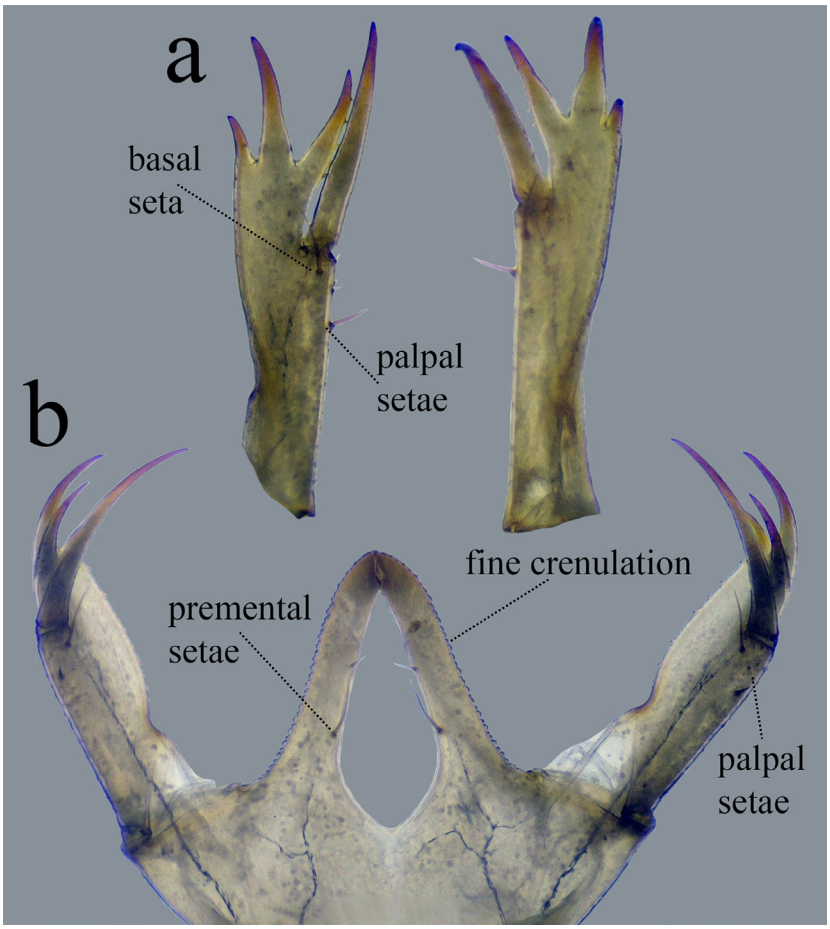

Figure 5. (a) Labial palps; (b) labial palps opened, and distal part of the prementum.

Table 1. Morphological characteristics of larvae of *Hetaerina* species occurring in the state of Minas Gerais. ? = Structure represented only in a dorsal view drawing in the original description.

Species	Described by	Posterolateral protuberances of head	Pronotal protuberances	N° premental setae	Lateral spines on abdominal segment 8	Mandible formula
Hetaerina auripennis (Burmeister, 1839)	Santos, 1970a	less prominent and rounded	as long as twice its basal width	1	present	_
Hetaerina brightwelli (Kirby, 1823)	Santos, 1972	lacking	as long as basal width (?)	2	absent	_
***H. giselae* sp. nov.**	**This study**	**prominent and rounded**	**half the length of the basal width**	**2**	**present**	**L 12345 0 a($m^{1,2,3,4,5,6}$)b/ R 12345 y a**
Hetaerina hebe Selys, 1853	Santos, 1970b	_	_	3	present	_
Hetaerina mendezi Jurzitza, 1982	von Ellenrieder, 2007	prominent and acute	as long as twice its basal width	1	present	L 1'12345 0 a(m^{1234-6}) b/ R 12345 y a b
Hetaerina rosea Selys, 1853	Pessacq & Muzón, 2004	less prominent and rounded	as long as basal width	1	present	L 12345 0 a($m^{1,2,3,4,5}$) b / R 12345 y a
Hetaerina rosea Selys, 1853	von Ellenrieder, 2007	less prominent and rounded	as long as basal width	1	present	L 1'12345 0 a(m^{1234-6})b/ R 12345y a b

Pronotum with lateral protuberances half the length of the basal width, slightly upturned (Figure 4c). The prothoracic supracoxal apophyses were blunt and weakly developed (Figure 4b). Wing pads reached the anterior margin of the third abdominal segment.

Abdomen. In the lateral view, the male gonapophysis does not extend beyond the sternum of S9 (Figure 6a); cercus conical and straight, 0.38 the length of S10 (Figure 6b); segment 10 with several spines. Caudal gills 5.9 (lateral), 4.3 (medial) longer than wide, with dentate margins, the lateral apex being acute, and the medial apex being oval (Figure 6c,d).

Measurements. AL: 8.7; Pfl: 2.6; MsfL: 4.2; MtfL: 5.2 ,TL: 15.7 (without gills).

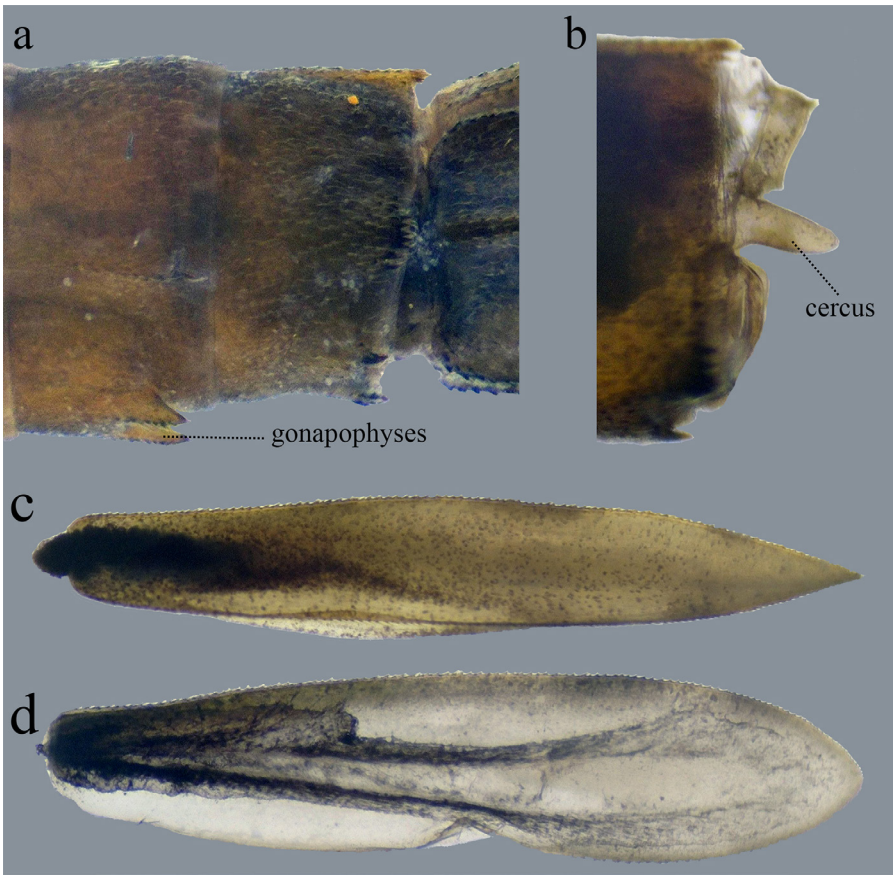

Figure 6. Male larval structures: (a) lateral view of gonapophysis; (b) cercus; (c) lateral caudal gill; (d) medial caudal gill.

Figure 7. Male resting on the marginal vegetation of Neném Waterfall, an important collection site for the park and for the new species.

Diagnosis

In the redescription of *H. rosea*, originally described by Pessacq & Muzón (2004), von Ellenrieder (2007) compiled several characters to distinguish the larvae of this group. Here, we used some of these characteristics, with the addition of mandibular formula, including the larvae of the newly described species (Table 1). The larvae of *Hetaerina* species occurring in the state of Minas Gerais differ in several aspects from the larva described in this study.

Habitat and biology

The study area is characterized by an area of rupestrian fields/rocky outcrop vegetation associated with the Atlantic Forest fragments. Adults and larvae were collected along the course of streams (1st order), waterfalls, and temporary ponds in the Pico do Itambé State Park at the following locations: Campina, Tropeiro's Trail, Água Santa Waterfall, Fumaça Waterfall, Neném Waterfall and Rio Vermelho Waterfall, locations where many adults were flying and resting on the marginal vegetation (Figure 7). The larvae were found on rocky slopes with less intense and calmer water flow, using angiosperms of the Podostemaceae family (identified by MSc. Glauco Oliveira) as support on the riverbed, as well as accumulations of fallen leaves for the same purpose.

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Supplementary Material

Supplementary Figure 1. Phylogenetic relationships of species in the Hetaerininae group based on the COI mitochondrial gene and the phylogenetic position of *Hetaerina giselae* sp. Bootstrap values above 50% are shown near the nodes.

Supplementary Table S1. Sequences used in phylogenetic analysis.